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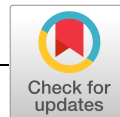
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REVIEW ARTICLE

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Activation of transcription factor Nrf2 to counteract mitochondrial dysfunction in Parkinson's disease

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Abstract

Parkinson's disease (PD) is a progressive neurodegenerative disorder, for which no disease-modifying therapies are available to date. Although understanding of the precise aetiology of PD is incomplete, it is clear that age, genetic predisposition and environmental stressors increase the risk. At the cellular level, oxidative stress, chronic neuroinflammation, mitochondrial dysfunction and aberrant protein aggregation have been implicated as contributing factors. These detrimental processes are counteracted by elaborate networks of cellular defence mechanisms, one of which is orchestrated by transcription factor nuclear factor-erythroid 2 p45-related factor 2 (Nrf2; gene name *NFE2L2*). A wealth of preclinical evidence suggests that Nrf2 activation is beneficial in cellular and animal models of PD. In this review, we summarise the current understanding of mitochondrial dysfunction in PD, the role of

Abbreviations: ARE, antioxidant response element; ATP, adenosine triphosphate; DMF, dimethyl fumarate; Drp1, dynamin-related protein 1; ETC, electron transport chain; GSK3, glycogen synthase kinase 3; HO-1, heme oxygenase-1; Keap1, Kelch-like ECH-associated protein 1; LRKK2, leucine-rich repeat kinase 2; Map1b, microtubule-associated protein 1b; Mfn, mitofusin; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NQO1, NAD(P)H:quinone oxidoreductase 1; Nrf2, nuclear factor-erythroid 2 p45-related factor 2; Opa1, optic atrophy 1; OXPHOS, oxidative phosphorylation; PD, Parkinson's disease; PGAM5, phosphoglycerate mutase family member 5; PGC1 α , peroxisome proliferator-activated receptor gamma coactivator 1 α ; PINK1, PTEN-induced putative kinase 1; PTEN, phosphatase and tensin homologue; ROS, reactive oxygen species; SOD, superoxide dismutase; tBHQ, *tert*-butyl-hydroquinone; TFAM, mitochondrial transcription factor A; β -TrCP, β -transducin repeats-containing protein.

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Nrf2 in mitochondrial function and explore the potential of Nrf2 as a therapeutic target for mitochondrial dysfunction in PD.

KEYWORDS

Nrf2, oxidative stress, Parkinson's disease

1 | INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative, progressive movement disorder, the prevalence of which is projected to double by 2030, reaching 9.3 million in the world's 10 most populous nations.¹ Pathologically, PD is characterised by the death of A9-type dopaminergic neurons in the substantia nigra, and the accumulation of proteinaceous aggregates in neurones.^{2,3} These aggregates are described as Lewy bodies, which are predominantly made up of α -synuclein protein. Degeneration of these dopaminergic neurones leads to deficit levels of dopamine, an essential neurotransmitter involved in the basal ganglia network regulating motor function.⁴ Clinically, patients experience a range of motor symptoms, collectively known as Parkinsonism, which include tremors, rigidity and bradykinesia.⁵ Non-motor symptoms include sleep disorders, apathy and pain.⁶ Currently, motor symptoms are treated with levodopa, dopamine agonists, monoamine oxidase-B inhibitors and catechol-O-methyl-transferase inhibitors as there are no disease-modifying therapies.⁷

There are two subtypes of PD, an idiopathic form and a familial genetic form.⁸ Seminal work in the past couple of decades has identified genes involved in the autosomal dominant and recessive forms of monogenetic PD.^{9–12} For example, genes encoding α -synuclein (*SNCA*) and leucine-rich repeat kinase 2 (*LRKK2*), respectively, were shown to cause autosomal dominant PD with features of Lewy pathology.^{11,13} Additionally, recessive autosomal mutations in *PINK1*, *PARKIN* and *DJ1* were shown to cause an earlier onset of Parkinsonism, but with slower progression independently of Lewy pathology.¹⁴ Many of these genes also have risk alleles for the development of idiopathic PD,^{15,16} suggesting the two subtypes could share similar underlying mechanisms of pathogenesis.

With the current understanding of the genes involved and the hallmarks of PD, several detrimental cellular processes have been implicated. For instance, oxidative stress, neuroinflammation, aberrant protein aggregation and mitochondrial dysfunction are all involved.^{8,17,18} These damaging processes are counteracted by induction of cytoprotective pathways. One prominent cytoprotective pathway is orchestrated by the transcription factor, nuclear factor erythroid 2 p45-related factor 2 (Nrf2).^{19–21} Nrf2 regulates the expression of several genes containing “antioxidant response elements” (AREs) in their promoters, which function to restore homeostasis after encountering electrophilic, inflammatory or oxidative stress.²² Recent findings strongly suggest that Nrf2 may have several significant roles in mitochondrial function,^{23,24} providing a potential therapeutic target for mitochondrial dysfunction in PD. The aim of this review is to summarise the current understanding on mitochondrial dysfunction in PD, the role of Nrf2 in mitochondrial function and ultimately explore its potential as a therapeutic target in PD.

2 | MITOCHONDRIAL HOMEOSTASIS

2.1 | Mitochondrial ROS signalling

Mitochondria are often described as cellular “power houses” due to their crucial function in synthesising adenosine triphosphate (ATP) through the process of oxidative phosphorylation (OXPHOS).²⁵ OXPHOS is

particularly important for neurones, which are highly metabolic and require high levels of ATP for neurotransmission and maintenance of ionic gradients across cell membranes.²⁶ Mitochondria are also one of the major contributors of reactive oxygen species (ROS) in the cell.^{27,28} Leakage of electrons at complex I and II of the electron transport chain (ETC), complex I damage and structural remodelling of mitochondrial supercomplexes, can cause incomplete reduction of oxygen, producing superoxide.^{29–32} However, mitochondria are equipped with superoxide dismutase 1 (SOD1) and 2 (SOD2) to prevent the potential damaging effects of superoxide. Activity of SOD1 and 2 cause superoxide to be converted into hydrogen peroxide in the mitochondrial intermembrane space and mitochondrial matrix, respectively. Unlike superoxide, hydrogen peroxide is relatively stable, making it a suitable signalling molecule. This is an important factor to highlight as several pathologies, including PD, report the detriments of excessive ROS, but ignore the significant role they play as signalling molecules in regulating cellular processes.³³ For example, mitochondrial ROS have been implicated in autophagy,³⁴ hypoxia³⁵ and immunity by activating toll-like receptor-initiated pathways.³⁶ More recently, it has been considered that exposure to ROS may actually stimulate health and wellbeing. Low levels of ROS, which do not tip cells into oxidative stress, are considered to be prime defence mechanisms via mitohormesis.³³ This process initiates adaptive responses which prepare and protect the cells from future insults.

2.2 | Fusion, fission and mitophagy

Mitochondria play fundamental roles in cell function and homeostasis, making mitochondrial quality control imperative. One way by which mitochondria maintain a healthy network and population is through the equilibrium of fission and fusion.³⁷ This is particularly important in neurones.^{38,39} Both fission and fusion are regulated by GTPase proteins such as dynamin-related protein 1 (Drp1), optic atrophy 1 (OPA1) and mitofusins 1 and (Mfn1 and Mfn2). In fission, mitochondria divide and Drp1 ultimately controls the splitting of the mitochondrial outer membrane to form two daughter organelles.⁴⁰ In fusion, both the outer and inner membranes of the mitochondria coordinately fuse with neighbouring mitochondria due to OPA1, Mfn1 and Mfn2 activity. These fusion–fission cycles are essential for maintaining a homogenous healthy mitochondrial population by ensuring that functional mitochondria are undergoing continuous exchange and mixing of content.⁴¹

Unhealthy or dysfunctional mitochondria, such as those with altered mitochondrial membrane potential, are unable to join the mitochondrial network through fusion and are consequently selected for degradation via mitophagy. Mitophagy is a process by which mitochondria are enveloped into double membrane autophagosomes (Figure 1). These then fuse with lysosomes ultimately leading to the degradation of their inner contents. The regulation and contribution of mitophagy to neurodegenerative diseases are topics of great interest with several ongoing investigations but many unanswered questions remain.⁴²

A well-studied mitophagy pathway is the PTEN-induced putative kinase 1 (PINK1) and Parkin pathway in which recessive mutations and risk alleles have been shown to be associated with genetic and idiopathic PD.^{14,16,43} In healthy conditions, PINK1 is integrated into the mitochondrial membrane and is cleaved on the N-terminus by mitochondrial proteases like presenilins-associated rhomboid-like protein.⁴⁴ The cleaved form of PINK1 is then degraded by ubiquitin mediated proteasome degradation upon its retranslocation from the mitochondrial membrane into the cytoplasm.⁴⁵ However, when mitochondria are damaged and an irreversible loss in mitochondrial membrane potential occurs, uncleaved PINK1 becomes stable on the mitochondrial membrane.⁴⁶ Stabilisation of full-length PINK1 allows it to phosphorylate several proteins, including ubiquitin and Parkin.⁴⁷ Furthermore, both phosphorylation events, one, at serine 65 located at the N-terminal ubiquitin-like domain of Parkin and another, at serine 65 of ubiquitin, are essential for full

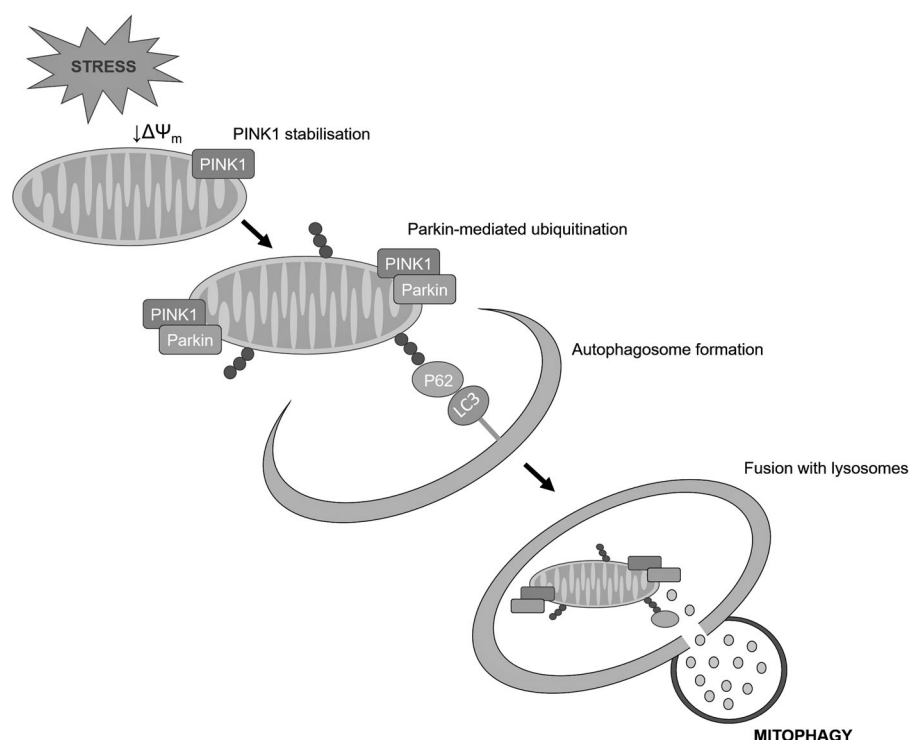


FIGURE 1 PTEN-induced putative kinase 1 (PINK1)–Parkin mediated mitophagy. When mitochondria are stressed or unhealthy, the mitochondrial membrane potential ($\Delta\Psi_m$) drops. This causes full length PINK1 to be stabilised on the mitochondrial membrane. The kinase activity of PINK1 causes it to autophosphorylate and phosphorylate ubiquitin and Parkin, which is then recruited to the outer mitochondrial membrane and ubiquitinates several outer mitochondrial membrane proteins. Such ubiquitin chains are then bound by mitophagic substrate adaptors like p62, which then bind to LC3-II to form an autophagosome. Here, damaged mitochondria are enclosed and fused with lysosomes for degradation

activation of Parkin,^{48–50} which ubiquitinates several outer mitochondrial membrane proteins, triggering mitophagy (Figure 1).

2.3 | Mitochondrial biogenesis

In coordination with mitophagy and the fusion–fission cycle, mitochondrial biogenesis also occurs. Mitochondrial biogenesis is defined as the “growth and division of preexisting mitochondria” which can be induced in response to several environmental factors such as oxidative stress, energy demand, exercise and differentiation.⁵¹ Although mitochondria contain their own genetic material, they rely on nuclear transcription and cytosolic translation of 1000–1500 proteins, which must be spatiotemporally regulated for correct mitochondrial biogenesis to occur. One way by which this is accomplished is through the major transcriptional regulator peroxisome proliferator-activated receptor gamma coactivator (PGC)1 α .⁵² PGC1 α induces nuclear respiratory factors 1 and 2 and binds to nuclear respiratory factor 1 to co-activate mitochondrial transcription factor A (TFAM) through promoter binding.⁵³ TFAM serves several functions to the mitochondria such as regulation of the initiation of mitochondrial DNA (mtDNA) transcription, mtDNA copy number and mtDNA packaging.⁵⁴ Interestingly, stimulation of $\alpha 7$ acetylcholine nicotinic receptor (nAChR)-mediated signalling increases mitochondrial biogenesis via Nrf2, heme oxygenase 1 (HO-1) and PGC1 α .⁵⁵

3 | MITOCHONDRIAL DYSFUNCTION IN PARKINSON'S DISEASE

3.1 | Complex I impairment

As neurones are highly dependent on the mitochondria for their metabolic and functional needs, it is no surprise that they are also very sensitive to mitochondrial dysfunction. Indeed, several neurodegenerative diseases are associated with mitochondrial dysfunction and oxidative stress.⁵⁶ More specifically, a substantial number of studies support the notion that mitochondrial dysfunction plays a significant role in the pathogenesis of PD. Early evidence was uncovered in the 1980s when several neurotoxins including rotenone, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and paraquat, were shown to induce Parkinsonism through inhibition of complex I in the ETC.^{57,58} These discoveries provided a core platform for the generation of various animal and cell models currently used to study idiopathic PD.⁵⁸ Further supporting the significance of complex I in PD was the finding that several subunits of complex I were decreased in the striata of postmortem brains of PD patients compared to controls, suggesting this to be a crucial factor in PD.^{59,60} Interestingly, α -synuclein, the fundamental component of Lewy bodies observed in PD, has also been shown to impair complex I in cell lines and transgenic mouse models.⁶¹

Although it is widely accepted that complex I has a role in PD pathology, the precise details are not fully understood. It is hypothesised that complex I inhibition causes alterations to the ETC activity which consequently affects the mitochondrial membrane potential and may, therefore, increase the level of ROS production, tipping cells into the state of oxidative stress. However, when Choi et al⁶² produced a transgenic complex I deficient *in vivo* model; dopaminergic neurones from this model were shown to have normal levels of ATP, ROS and oxygen consumption. Moreover, increased sensitivity to rotenone was also observed in these dopaminergic neurones. This is surprising and suggests that other intrinsic factors have a role in dopaminergic neuron vulnerability in PD.

Notably, the ETC proteins can form dynamic supercomplexes, the assembly of which provides an adaptive mechanism to varying carbon sources.⁶³ Studies in mitochondria from fibroblasts of patients with mutations in PINK1 have found that the levels of free complex I, and the ratio of free versus supercomplexes-bound complex I, are decreased in PD. Although the levels of free complex III are not affected, the ratio of free versus supercomplexes-bound complex III is decreased, and complex IV is significantly diminished.³¹ These findings suggest that structural remodelling of mitochondrial supercomplexes could be an important contributor to the bioenergetics deficits in PD.

3.2 | Mutations in PD impinging on mitochondrial homeostasis and function

The strongest line of evidence for mitochondrial dysfunction in PD was provided by the influential discoveries of PD-associated genes, which have been shown to directly or indirectly interfere with mitochondrial function. Although PINK1 is not required for basal mitophagy, even in tissues of high metabolic demand,⁶⁴ PINK1 (also known as PARK6) mutations or silencing leads to dysfunctional mitophagy,^{65,66} increased sensitivity to oxidative stress,⁶⁷ defective mitochondrial calcium regulation,^{67–69} impaired respiration and impaired ETC substrate availability in a range of PD models. More recently, PINK1 has also been shown to protect cells from α -synuclein induced cytotoxicity by preventing its localisation to the mitochondria and targeting it for autophagy.⁷⁰ Generally, PINK1 mutations, which are causatively associated with autosomal recessive PD⁷¹ lead to loss of function in PINK1,^{72,73} which in turn, may cause bioenergetic dysfunction in neurones.⁷⁴

Parkin (also known as PARK2), an E3 ubiquitin ligase, is also found to be mutated in PD and acts downstream of PINK1 in mitophagy.⁷⁵ Parkin has been reported to protect dopaminergic neurones against mitochondrial stress.⁷⁶ The actual mechanisms by which this occurs have not been fully elucidated, but the PINK1–Parkin pathway was shown to be activated.

LRRK2 mutations cause autosomal dominant familial PD. Several studies have shown that LRRK2 mutations have detrimental effects on the mitochondria. For example, mutant or kinase-dead LRRK2 mediates weaker protection in nematodes (expressing neuronal human LRRK2) to rotenone or paraquat, relative to the wild-type LRRK2.⁷⁷ Wang et al.⁷⁸ also showed the importance of LRRK2 in regulating mitochondrial dynamics through Drp1. Moreover, in induced pluripotent stem cell-derived neuronal cells from PD patients, LRRK2 mutations were shown to cause DNA mitochondrial damage.⁷⁹

A recent study suggests that there is crosstalk between LRRK2 signalling and PINK1-regulated mitochondrial homeostasis.⁸⁰ Activation of PINK1 indirectly induces the phosphorylation of the small GTPase Rab8A at serine 111,⁸¹ whereas LRRK2 mediates the phosphorylation of Rab8A at threonine 72.^{82,83} Interestingly, serine 111 phosphorylation prevents threonine 72 phosphorylation by LRRK2 and impairs the interactions of Rab8A with its cognate guanine nucleotide exchange factor and GTPase activating protein.⁸⁰

Mutations in DJ1 (also known as PARK7) are also reported in familial PD.⁸⁴ Loss of DJ1 causes mitochondrial depolarisation, fragmentation and increased autophagy.⁸⁵ Interestingly, the authors showed that DJ1 is involved in the maintenance of mitochondrial function during oxidative stress. This supports the understanding that DJ1 can stabilise Nrf2 under such conditions.⁸⁶ In addition, DJ1 was shown to be protective in the absence of PINK1, suggesting its neuroprotective effects are parallel to PINK1.⁸⁷

4 | NRF2

Nrf2 is a basic leucine zipper transcription factor which regulates the gene expression of a battery of cytoprotective proteins involved in numerous cellular processes, including ROS scavenging, xenobiotic metabolism and detoxification, glutathione and nicotinamide adenine dinucleotide phosphate (NADPH) homeostasis, and autophagy.^{19–21} In homeostatic conditions, Nrf2 is continuously targeted for ubiquitin-dependent proteasomal degradation by several negative regulatory complexes. The most studied negative regulator of Nrf2 is the Cullin 3/Ring-box1 E3 ubiquitin ligase complex substrate adaptor protein Kelch-like ECH-associated protein 1 (Keap1). Homodimeric Keap1 binds to the Nrf2-ECH homology (Neh) 2 domain of one Nrf2 molecule through two motifs, the lower affinity DLG motif and the higher affinity ETGE motif (Figure 2). Once bound, Nrf2 is correctly positioned for ubiquitination by Cullin 3 at the seven lysine residues present between the two motifs. Consequently, Nrf2 is then targeted for 26S proteasome degradation and, therefore, kept in low abundance within the cell. Nrf2 can also be regulated by another ubiquitin dependent pathway via the GSK3/β-TrCP/Cul1 (glycogen synthase kinase 3/β-transducin repeat-containing protein/Cullin 1) complex which, following phosphorylation, induces Nrf2 degradation through the Neh 6 domain (Figure 2).^{88,89}

In environments of oxidative and electrophilic stress, the degradation of Nrf2 is disrupted. This is due to the chemical modification (e.g., oxidation, alkylation) of cysteine residues in Keap1 that serve as sensors for electrophiles and oxidants.⁹⁰ Such modifications alter the substrate adaptor function of Keap1 and renders it unable to target Nrf2 for degradation.⁹¹ Therefore, Nrf2 remains bound to Keap1, saturating available Keap1 homodimers and allowing newly synthesised Nrf2 to translocate to the nucleus to dimerize with small musculoaponeurotic fibrosarcoma (sMaf) proteins for ARE-mediated target gene transcription. Notably, although we focus on the Keap1-mediated regulation of Nrf2 in this review, disruption of the Nrf2 degradation through both GSK3/β-TrCP/Cul1 and Keap1/Cul3 pathways has been shown to be beneficial in models of PD.

5 | NRF2 IN PD

The published literature strongly suggests that Nrf2 has a protective role in PD.⁹² A meta-analysis of nine PD microarray datasets identified 31 common downregulated genes containing the ARE consensus sequence, despite increased levels of Nrf2,⁹³ suggesting that Nrf2 signalling may be impaired. Moreover, the Nrf2-target proteins

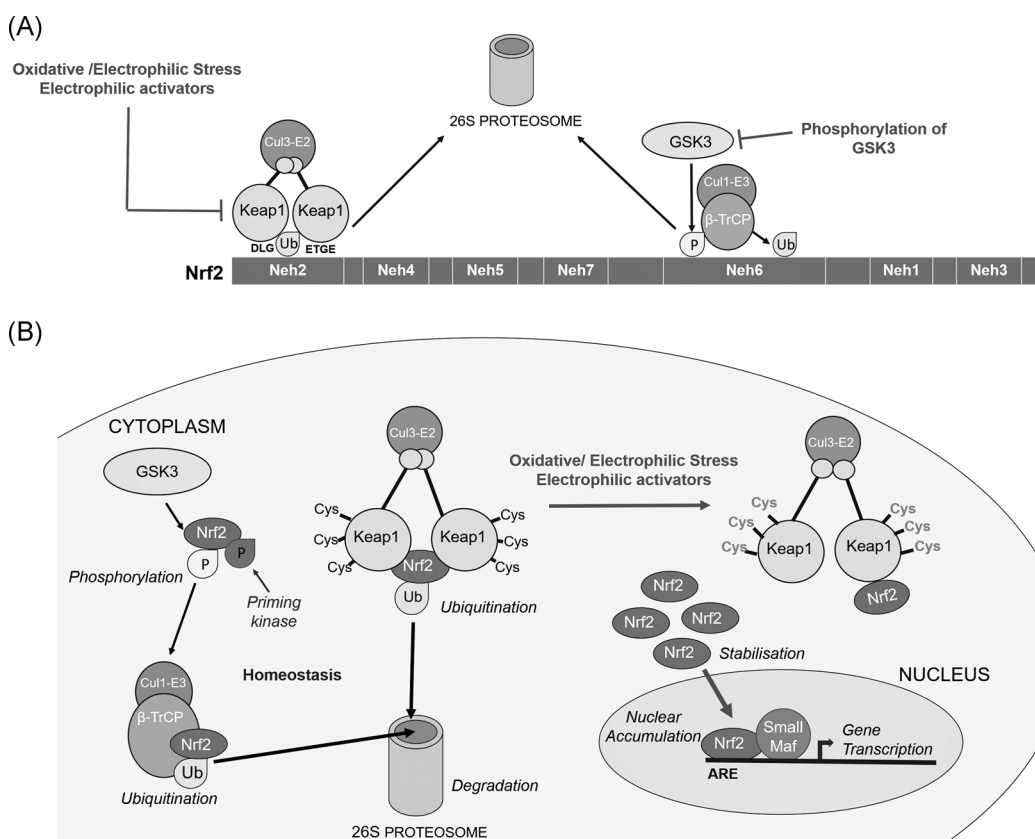


FIGURE 2 Regulation of Nrf2. (A) In homeostatic conditions, Nrf2 is targeted for proteasome degradation through GSK3/β-TrCP/Cul1 and Keap1/Cul3 via the Neh6 and Neh2 domains, respectively. Keap1 binds to the DLG and ETGE motifs in the Neh2 domain of Nrf2. Keap1 recruits the Cullin 3 complex, which ubiquitinates seven lysine residues found between the two motifs. In electrophilic/oxidative stress or exposure to electrophilic activators of Nrf2, Keap1 is modified and unable to target Nrf2 for degradation. Additionally, Nrf2 can be phosphorylated by GSK3, which recruits the β-TrCP/Cullin 1 complex. This complex can also ubiquitinate Nrf2 for proteasome degradation. Phosphorylation of GSK3, for example by Akt, causes it to become inactive. Here, it is unable to phosphorylate Nrf2 at the Neh6 domain and, therefore, prevents β-TrCP/Cullin 1 dependent degradation. (B) In oxidative/electrophilic stress or exposure to electrophilic Nrf2 activators, Keap1 is unable to target Nrf2 for degradation. This causes newly synthesised Nrf2 to stabilise in the cytoplasm and translocate to the nucleus. In the nucleus, Nrf2 binds to small Maf (sMaf) proteins and together they bind to antioxidant response elements (ARE) found in the promoters of Nrf2 target genes to initiate gene transcription. GSK3/β-TrCP/Cul1, glycogen synthase kinase 3/β-transducin repeat-containing protein/Cullin 1; Keap1, Kelch-like ECH-associated protein 1; Nrf2, nuclear factor-erythroid 2 p45-related factor 2

NAD(P)H:quinone oxidoreductase 1 and p62/sequestome-1 (SQSTM1) were partly sequestered in Lewy bodies in post-mortem samples of PD patients.⁹⁴ Nrf2 signalling is also impaired in human A9-type dopaminergic neurones expressing mutant *SNCA*. Low Nrf2 activity resulted in reduced expression of microtubule-associated protein (Map1b), which in turn caused neuritic defects that could be rescued by Nrf2 activation.⁹⁵ Conversely, a single-nucleotide polymorphism within the regulatory region of the *MAPT* gene (encoding microtubule-associated protein Tau), which is consistently occupied by Nrf2/sMaf, was associated with a highly protective allele that had been identified in multiple genome-wide association studies of PD.⁹⁶ Additionally, a haplotype of the *NFE2L2* gene (encoding Nrf2) that includes a promoter polymorphism resulting in enhanced Nrf2 transcriptional activity was

shown to be associated with a reduced risk for or a later onset of PD.⁹⁷ Four independent European and one Australian case-control studies have supported this initial finding.^{98,99} By contrast, no association of such polymorphisms with PD was observed in a Taiwanese population,¹⁰⁰ indicating that differences in ethnicity and/or environment are important determinants of disease susceptibility. The role of environmental factors is further supported by a study, which concluded that common *NFE2L2* variants could be particularly important in reducing PD susceptibility under conditions of pesticide exposure.⁹⁹

6 | NRF2 AND MITOCHONDRIAL HEALTH

6.1 | Nrf2 and oxidative phosphorylation

The role of Nrf2 in the maintenance of overall cellular redox homeostasis is well accepted in the field. More recent evidence suggests that Nrf2 may also have a perquisite role in mitochondrial structure, function and integrity.^{23,24} More specifically, Nrf2 has a critical role in the regulation of mitochondrial respiration and redox homeostasis.¹⁰¹ In isolated Nrf2-knockout (Nrf2-KO) mouse embryonic fibroblast cells and primary cultured neurones, the basal mitochondrial membrane potential, oxygen consumption rate and ATP levels were lower in comparison to their wild-type counterparts. Moreover, by using OXPHOS and glycolysis inhibitors, it was demonstrated that Nrf2 alters the way by which ATP is produced in cells. When wild-type neurones were treated with the complex V inhibitor, oligomycin, a dramatic decline in ATP production was observed. This was not altered when iodoacetic acid (an inhibitor of glycolysis) was added to the cells, suggesting glycolysis is not involved in the production of ATP in these cells. Conversely, in Nrf2-KO cells treated with oligomycin, an increase in ATP production was described. Interestingly, ATP was completely depleted with the addition of iodoacetic acid, suggesting that in Nrf2-KO cells, glycolysis is the main source of ATP and not OXPHOS. In addition, the increase in ATP upon oligomycin addition shows that complex V, which usually functions as an ATP-synthase in OXPHOS, is actually functioning as an ATP-ase. It is likely that this switch in activity is to maintain the mitochondrial membrane potential required for mitochondrial integrity. The rate of regeneration of both NADH and FADH₂ (complex II substrate) were also slower in the Nrf2-KO cells.¹⁰¹

6.2 | Nrf2 and mitophagy

A significant proportion of the literature highlights the importance of Nrf2 in mitochondrial integrity. Nrf2 has a range of downstream target genes, which are involved in selective autophagy. For example, p62/and autophagy-related gene 8 (ATG8).^{102–104} p62 is an important player in PINK1–Parkin mitophagy¹⁰⁵ and has been shown to have roles in the maintenance of the mitochondrial membrane potential and bioenergetics (Figure 1).^{106,107} Moreover, in p62-KO cells, a similar mitochondrial phenotype to Nrf2-KO was observed.^{101,107} Pharmacological activation of Nrf2 with RTA-408, sulforaphane or TBE-31 in these cells, increased complex I substrate NADH and restored the mitochondrial membrane potential.¹⁰⁷ It is clear that both Nrf2 and p62 are important in mitochondrial bioenergetics. p62 is also considered to be important in mitophagy but whether this is regulated by Nrf2 remains to be elucidated.

As previously mentioned, PINK1 plays a prominent role in the quality control of mitochondria by initiating the process of mitophagy in unhealthy damaged mitochondria. Interestingly, PINK1 has also been shown to have four potential ARE sequences in its promoter, suggesting that PINK1 is regulated by Nrf2. Indeed, it was shown that pharmacological induction of Nrf2, for example by *tert*-butyl-hydroquinone (tBHQ), caused increased PINK1 protein expression and messenger RNA (mRNA) levels, which was lost when validated Nrf2 small interfering RNAs (siRNAs) were used.¹⁰⁸ These inducers increased hydrogen peroxide levels, and co-treatment of

them with antioxidant *N*-acetylcysteine abolished the induction of PINK1, suggesting the Nrf2–PINK1 axis is dependent on ROS. Further supporting the link between Nrf2 and PINK1, is the effect of tomatidine in the induction of mitophagy in *Caenorhabditis elegans* (*C. elegans*) via SKN-1 (Nrf2 homologue).¹⁰⁹ Tomatidine, abundantly found in unripen tomatoes, induced mitophagy through DCT-1 (PINK1 homologue) and similar to Murata et al.,¹⁰⁸ it was proposed that ROS are key to this process. Low levels of ROS induced by tomatidine are thought to activate Nrf2, which ultimately leads to mitophagy. However, in this study, the knockout (KO) or knockdown (KD) effect of Nrf2/SKN-1 was not shown and, therefore, other pathways may be involved. This is particularly important to establish in view of the fact that SKN-1 is even more closely related to the endoplasmic reticulum-residing transcription factor nuclear factor-erythroid 2 p45-related factor 1 (Nrf1, gene name *NFE2L1*, not to be confused with nuclear respiratory factor 1), which like Nrf2, controls ARE-mediated transcription.¹¹⁰ Overall, emerging evidence suggests that Nrf2 might be an important mediator of PINK1-induced mitophagy and, therefore, overall mitochondrial integrity.

It has been suggested that PINK1 can also influence Nrf2 activity and expression.¹¹¹ In a model of ubiquitin proteasome system dysfunction, mutant PINK1 (G309S) inhibited heme-oxygenase 1 (HO-1) expression, an Nrf2 target gene, in SH-SY5Y cells.¹¹¹ Moreover, in this MG132-induced model, Nrf2 nuclear translocation, protein and mRNA levels were antagonised by PINK1 G309D. First, this suggests that PINK1 has a role in regulating Nrf2 transcriptional activity. Second, the location of this mutation on PINK1 could be responsible for Nrf2 suppression. This missense mutation is known to cause PD, possibly through impairment of PINK1 kinase activity, substrate recognition or defects in complex I.^{111,112} Together, these findings suggest that Nrf2 may potentially require PINK1-dependent signalling to mediate its cytoprotective effects through downstream targets on mitochondria independently of mitophagy (Figure 3).

A population of Nrf2 has been shown to be tethered to the mitochondria in a quaternary complex with a Keap1 dimer though the mitochondrial serine/threonine protein phosphatase PGAM family member 5 (PGAM5).¹¹³ The actual role of this complex is not clearly defined. However, knockdown of PGAM5 debilitated PINK1-induced mitophagy in vitro, led to degeneration of dopaminergic neurones and induced Parkinson-like movement phenotype in mice.¹¹⁴ The same study showed that PGAM5, through an evolutionary conserved region (amino acids 98–110), directly binds and stabilises wild-type, but not PD-associated mutant PINK1. The role of Nrf2 within the PGAM5–Nrf2–Keap1 complex on mitophagy is yet to be studied.¹¹⁵ Collectively, based on the findings from the work of Murata et al.¹⁰⁸ and Lu et al.,¹¹⁴ it could be proposed that the PGAM5–Nrf2–Keap1 complex may function with Keap1 as an immediate mitochondrial ROS sensor which allows newly synthesised Nrf2 to accumulate and induce PINK1 expression. PINK1 is then stabilised by PGAM5 in the complex to initiate mitophagy (Figure 3).

Notably, recent studies have uncovered a PINK1/Parkin-independent mitophagy pathway mediated by p62, Keap1 and Rbx1, where Keap1 and Rbx1 are recruited to p62, promoting mitochondrial ubiquitination.^{116,117} Inhibiting the interaction of p62 with Keap1 prevents p62-mediated mitochondrial ubiquitination.

6.3 | Nrf2 and mitochondrial trafficking

In another context, knockdown of Nrf2 in the Nrf2–PGAM5–Keap1 complex hindered mitochondrial retrograde trafficking in induced proteasome inhibition.¹¹⁵ This was due to aberrant degradation of Miro2 by unconstrained Keap1–Cullin 3 activity. Miro2 is a mitochondrial outer membrane Rho GTPase involved in physically linking mitochondria to microtubules for trafficking. Here, Nrf2 serves a nontranscriptional role in keeping Keap1 occupied, preventing Miro2 degradation and allowing mitochondrial retrograde trafficking, which is of particular importance for neurones. However, an attempt to coimmunoprecipitate Keap1 and Miro2 did not detect binding, suggests that this interaction may be indirect and other proteins and complexes may be involved.

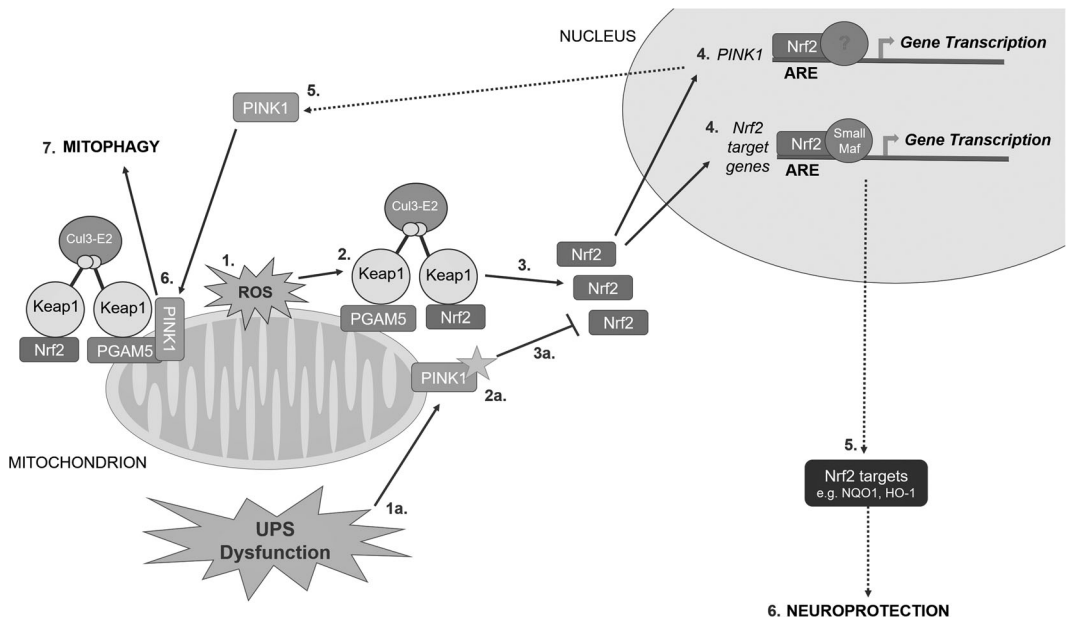


FIGURE 3 Hypothetical PINK1-Nrf2 axis. (1) Reactive oxygen species (ROS) from the mitochondria (or other cellular components) modify Keap1 in the PGAM5-Nrf2-Keap1 quaternary complex (2), potentially preventing Nrf2 turnover. This may allow Nrf2 levels to stabilise in the cytoplasm (3) and translocate to the nucleus for transcription of Nrf2-dependent genes, including NAD(P)H:quinone oxidoreductase 1 (NQO1) and heme oxygenase-1 (HO-1), as well as PINK1 (4) and subsequent translation (5). The resulting proteins are the actual protectors (6). PGAM5 can then bind and facilitate the stabilisation of PINK1 on the mitochondrial membrane, preventing its cleavage from presenilins-associated rhomboid-like protein. This stabilisation then initiates the process of mitophagy (7), essential for the maintenance of a healthy mitochondrial population. In conditions of ubiquitin-proteasome system dysfunction (1a), also reported in PD, mutant PINK1 (2a) may inhibit Nrf2 mRNA and/or protein levels, its nuclear translocation or transcriptional activity (3a). Such effects may inhibit the expression of Nrf2 target genes, depriving the cell from this cytoprotective mechanism. mRNA, messenger RNA; Nrf2, nuclear factor-erythroid 2 p45-related factor 2; PD, Parkinson's Disease; PGAM5, phosphoglycerate mutase family member 5; PINK1, PTEN-induced putative kinase 1; UPS, ubiquitin proteasome system

6.4 | Nrf2 and mitochondrial biogenesis

ARE sequences have been reported in the promoters of PGC1 α and nuclear respiratory factor 1, both of which are essential for mitochondrial biogenesis, suggesting that Nrf2 regulates their expression.^{108,118} More specifically, in mouse heart, HO-1 induced Nrf2 activation, which in turn upregulated nuclear respiratory factor 1 on both the protein and mRNA level. This ultimately protected cells from the cardiotoxin, doxorubicin, through increased mitochondrial biogenesis.¹¹⁸ Further supporting the role of Nrf2 in mitochondrial biogenesis, tomatidine, which activates Nrf2, was also shown to increase mitochondrial content, mitochondrial membrane potential and cellular ROS in *C. elegans* and primary rat cortical neurones.¹⁰⁹ Additionally, a concentration dependent increase in SOD2, complex IV-COX II, COX-VI and heat shock protein 60 mitochondrial proteins were observed in human neural cells upon treatment with tomatidine. Furthermore, Merry and Ristow¹¹⁹ showed that in addition to nuclear respiratory factor 1 and antioxidant enzymes like SOD2, TFAM was also upregulated by Nrf2 in skeletal muscle post-acute exercise. Considerable evidence proposes Nrf2 to have a direct role in mitochondrial biogenesis through upregulation of important transcription factors across a range of tissues. However, limited studies focus on the importance of Nrf2 and its mechanisms in neuronal mitochondrial biogenesis. Indeed, Fang et al¹⁰⁹ address this to some extent but do not establish whether the tomatidine-induced biogenesis is due to Nrf2 alone.

6.5 | Nrf2 in mitochondrial dynamics

When mitochondria are stressed, they form a hyperfused network, which allows them to reduce ROS production, increase ATP production and develop resistance against apoptotic signals. Drp1 activity blocks mitochondrial recruitment and fission, but Nrf2 promotes the degradation of Drp1 allowing this hyperfusion response to occur.¹²⁰ In this study, Nrf2 overexpression reduced Drp1 levels and increased hyperfusion. This was further supported with Nrf2 pharmacological activation in rat hippocampi in vivo and in murine primary neurones and fibroblasts. It was also shown that Nrf2 transcription was required for this effect on Drp1. Moreover, with proteasome inhibition, Drp1 levels increased despite Nrf2 levels being elevated. This suggests that Nrf2-dependent upregulation of proteasome activity may cause decreased stability of Drp1 and hence hyperfusion in stress. Interestingly, this response was shown to be independent of Keap1. In a Huntington's disease model in immortalized murine striatal cell lines, both fusion and Nrf2 signalling were impaired,¹²¹ which is consistent with muted Nrf2 activity in neural stem cells from Huntington's disease patients.¹²² Interestingly, the Nrf2 inducer sulforaphane promotes hyperfusion, but independently of Nrf2. Sulforaphane mediated its effects despite siRNA-induced KD of Nrf2 or Keap1 in RPE-1 cells and was shown to prevent Drp1 localising and accumulating at the mitochondria.¹¹⁸

7 | TARGETING NRF2 IN MITOCHONDRIAL DYSFUNCTION IN PD

A large volume of preclinical experimental evidence supports the human data suggesting that Nrf2 has a protective role in PD. Thus, the expression of human α -synuclein in the ventral midbrain of Nrf2-deficient mice leads to degeneration of nigral dopaminergic neurons and increased dystrophic dendrites, reminiscent of Lewy neurites; this neuronal loss is associated with neuroinflammation and gliosis.¹²³ Nrf2-deficient mice are also much more sensitive to the neurotoxic effects of MPTP than their wild-type counterparts, whereas Nrf2 activation has neuroprotective effects in Nrf2-wild-type, but not Nrf2-deficient mice in this model of PD,^{124–130} as well as in mice expressing human α -synuclein.⁹⁴ In both models, these protective effects are associated with a decrease in oxidative damage and neuroinflammation. Nrf2 activation protects both neurones and astrocytes. Thus, astrocytes from adult rats are more susceptible to mitochondrial toxicity caused by the MPTP metabolite 1-methyl-4-phenylpyridinium (MPP+) than astrocytes from newborn rats, but importantly, are protected by the Nrf2 activator tBHQ.¹³¹ Grafting primary postnatal astrocytes above the substantia nigra of aged MPTP-treated mice after the onset of motor symptoms led to Nrf2 activation and counteracted the motor deficits.¹³² In a rotenone-induced PD mouse model, Nrf2 activation increased the levels of glutathione and lowered lipid peroxidation in the striatum and improved motor dysfunction.¹³³ In addition to its protective effects against oxidative stress and inflammation, Nrf2 activation protects against PD-associated protein toxicity. A longitudinal imaging platform has been developed to visualize the metabolism and location of mutant LRRK2 and α -synuclein in living primary rat neurons at the single-cell level.¹³⁴ Using this platform, these researchers found that Nrf2 reduced the toxicity of these proteins by accelerating the degradation of α -synuclein and sequestering misfolded diffuse LRRK2 into insoluble inclusion bodies.

Although many studies have explored the potential of Nrf2 inducers in PD,^{135–137} few have considered how they may affect mitochondrial dysfunction in PD.¹³⁸ Interestingly, Nrf2 and PINK1 deficiencies have similar effects on mitochondrial bioenergetics. For example, similar to that of Nrf2 deficiency, it was shown that provisions of complex I and II substrates to PINK1-deficient cells led to recovery of the mitochondrial membrane potential and mitochondrial respiration.^{68,139} This ultimately reduced the sensitivity of neurones to dopamine-induced toxicity. Such similarities between Nrf2 and PINK1 deficiencies suggest that activation of Nrf2 may potentially rescue mitochondrial respiration in PINK1-deficient cells and vice versa. Indeed, the mitochondrial membrane potential was restored in primary murine PINK1-KO co-cultures of neurones and astrocytes when exposed to the pentacyclic cyanoenone triterpenoid Nrf2 inducer RTA-408.¹³⁹ Similar effects were seen with the isothiocyanate sulforaphane, a potent naturally occurring Nrf2 inducer.¹⁴⁰ More importantly, these Nrf2 inducers were also able to

significantly reduce cell death in dopamine-exposed PINK1-KO astro-neural co-cultures. Notably, RTA-408 was also shown to rescue the bioenergetics deficits, preserve the hippocampal neurons and astrocytes and dramatically reduce the frequency of late spontaneous seizures in a rat model of epilepsy.^{141,142} Sulforaphane had a similar effect.¹⁴³ Here, Nrf2 inducers are illustrated to overcome mitochondrial dysfunctions, further supporting the potential role of Nrf2 as a therapeutic target for PD. Indeed, the protective effects of sulforaphane have been demonstrated in a number of PD mouse models. Thus, in the MPTP mouse model, administration of sulforaphane increases Nrf2-target gene expression in striatum and ventral midbrain, decreases the levels of proinflammatory mediators and reduces the loss of dopaminergic neurons, astrogliosis and microgliosis; importantly, these effects are not observed in Nrf2-KO mice.^{126,127,129} Dietary intake of glucoraphanin, the biogenic precursor of sulforaphane, is also protective.¹⁴⁴ Similarly, in mouse models of 6-dihydroxy-dopamine- or rotenone-induced neurotoxicity, sulforaphane treatment enhances Nrf2-target gene expression and reduces macromolecular damage, degeneration of dopaminergic neurons and motor function deficits.^{145–147} In view of the convincing experimental evidence from these animal models and considering that sulforaphane has been and currently is in multiple clinical trials,^{21,148} it will be important to investigate its therapeutic potential in PD patients.

Dimethyl fumarate (DMF; trade names Tecfidera®, Skilarence®) is the only Nrf2 inducer which is currently in clinical practice and is used for treatment of multiple sclerosis and moderate to severe plaque psoriasis.^{21,149,150} The potential of DMF and its active metabolite monomethylfumarate on Nrf2-induced neuroprotection have been compared in an MPTP-induced PD model.¹⁵¹ It was shown that with increasing doses of DMF, there was increased glutathione depletion, reductions in cell viability and inhibition of both mitochondrial oxygen consumption and glycolysis. However, the opposite was observed with MMP, which caused increases in all of these parameters. Despite their differences, both upregulated mitochondrial biogenesis in an Nrf2-dependent manner. In vivo, both blocked MPTP-induced neurotoxicity in wild-type, but not in Nrf2-KO mice. This was shown to be due to the Nrf2 effect on several cellular processes, including mitochondrial function and biogenesis. Overall, both compounds were shown to have neuroprotective effects.¹⁵¹ This study illustrates that targeting Nrf2 has the potential to ameliorate mitochondrial dysfunction and other detrimental processes, such as neuroinflammation and oxidative stress, which are also involved in PD.

A non-electrophilic Nrf2 activator called p62-mediated mitophagy inducer (PMI), which disrupts the protein–protein interactions between Keap1 and Nrf2,¹⁵² has been shown to drive mitophagy without dissipating the mitochondrial membrane potential or Parkin recruitment.¹⁵³ PMI induces mitochondrial respiration and the expression of p62.¹⁵⁴ Similarly to PMI, other Keap1-Nrf2 protein-protein interaction inhibitors also induce mitophagy.¹⁵⁴ However electrophilic Nrf2 inducers, such as sulforaphane and DMF, which covalently modify Keap1, are unable to induce such response,¹⁵⁴ even though Nrf2 regulates the expression of several autophagy-related genes,^{155,156} and its activation by sulforaphane and other electrophilic inducers promotes autophagy.^{157,158} In fact, co-treatment of PMI with sulforaphane was shown to inhibit PMI-induced effects like the accumulation of p62 required for mitophagy.¹⁵⁴ Yet, both electrophilic and non-electrophilic Nrf2 activators induce similar alterations on mitochondrial morphology and bioenergetic profiles, suggesting that the reversible inhibition of the Keap1-Nrf2 protein–protein interactions by PMI is specifically important for mitophagy.¹⁵⁴ Most importantly, the PINK1-Parkin pathway was not required for mitophagy induced by PMI.^{153,154,159} This finding is particularly exciting as it suggests that non-electrophilic compounds that target Keap1 can be used to rescue mitochondrial turnover without relying on the PINK1-Parkin pathway, which is often defective in PD.

8 | OUTLOOK

Growing experimental evidence strongly places mitochondrial dysfunction as a prominent feature in PD, making it a possible therapeutic target. With no available therapies and the substantial evidence supporting Nrf2 signalling in mitochondrial homeostasis and integrity, targeting the Keap1-Nrf2 pathway provides an exciting therapeutic

strategy for PD. A large volume of research indicates that Nrf2 plays a significant role in the maintenance of mitochondrial health, including the mitochondrial membrane potential, respiration, mitophagy and mitochondrial biogenesis. Whether these functions are all met by Nrf2 in the PD brain remains to be further explored. However, promising preclinical evidence already shows the protective effect of pharmacological Nrf2 activation in models of PD. Further unravelling of the underlying mechanisms by which Nrf2 contributes to the maintenance of mitochondrial homeostasis in PD may lead to promising new therapies.

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